J. Clin. Chem. Clin. Biochem.
Vol. 17, 1979, pp. 689–691

Influence of Indicating Enzyme Reaction on Apparent Creatine Kinase Activity

Creatine Kinase in Serum, VII.

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(Received September 3, 1979)

Summary: Comparison of the indicative systems yeast glucose-6-phosphate dehydrogenase/$NADP^+$, *leuconostoc* glucose-6-phosphate dehydrogenase/$NADP^+$ and *leuconostoc* glucose-6-phosphate dehydrogenase/NAD^+ showed excellent correlation and no differences in apparent creatine kinase activity with the two methods using $NADP^+$. By using NAD^+ with the *leuconostoc* enzyme relative recovery of apparent creatine kinase activity is lower due to interference of other serum constituents. The mean value of the relative differences versus methods using $NADP^+$ was 5.8% in our experiments.

Abhängigkeit der Kreatinkinase-Aktivität vom gewählten Indikator-System

Kreatinkinase-Aktivität im Serum, 7. Mitteilung

Zusammenfassung: Der Vergleich der Indikator-Systeme Hefe-Glucose-6-phosphat-dehydrogenase/$NADP^+$, *Leuconostoc*-Glucose-6-phosphat-dehydrogenase/$NADP^+$ und *Leuconostoc*-Glucose-6-phosphat-dehydrogenase/NAD^+ zeigte für die zwei Indikator-Systeme mit $NADP^+$ eine vorzügliche Korrelation zwischen den Methoden und keine methodischen Differenzen bezüglich der Kreatinkinase-Aktivität. Bei Verwendung des *Leuconostoc*-Glucose-6-phosphat-dehydrogenase/NAD^+-Systems wird im Vergleich zum Hefe-Glucose-6-phosphat-dehydrogenase/$NADP^+$-System eine geringere Kreatinkinase-Aktivität gefunden. Dies wird auf den Einfluß anderer Serumbestandteile zurückgeführt. Nach unseren Ergebnissen findet im Mittel das $NADP^+$-System 5,8% mehr.

Introduction

In a series of publications *Gábor Szász* (1–6) submitted detailed information on the background of the studies on creatine kinase activity determination in serum leading to common recommendations of some national societies for Clinical Chemistry. (7–11) When *Gábor Szász* died a cooperative study on the influence of different indicating systems was running in Gießen, Bern and Tutzing. The results reported here complete the series, together with an review on the reasons for the selection of N-acetyl-cysteine as reactivating thiol to be published in Clinical Chemistry.

Coenzyme specificities of glucose-6-phosphate dehydrogenase preparations from yeast and *leuconostoc* species are different (12). The yeast enzyme is strongly specific for $NADP^+$ whereas the *leuconostoc* enzyme can use both $NADP^+$ and NAD^+, the latter with lower affinity (13). As most dehydrogenases which might be present in human sera with higher activities are specific toward NAD^+/NADH, enhanced interference was expected sub-

0340-076X/79/0017-0689$2.00

stituting $NADP^+$ by NAD^+ in creatine kinase assays (14,15).

Materials and Methods

Adenosine diphosphate, adenosine monophosphate, diadenosine pentaphosphate, N-acetyl-cysteine, creatine phosphate, glucose and hexokinase were dissolved to a common stock solution of 10% higher concentrations as compared to the recommended methods in imidazole buffer pH 6,7 containing 2 mmol/l EDTA. To each 90 ml of this stock solution were added:

for method 1:

0,438 g $NADP^+$, disodium salt and 440 U yeast glucose-6-phosphate dehydrogenase

for method 2:

0,438 g $NADP^+$ disodium salt and 440 U *leuconostoc* glucose-6-phosphate dehydrogenase.

for method 3:

0,354 g NAD^+, free acid and 440 U *leuconostoc* glucose-6-phosphate dehydrogenase.

These assay solutions were brought to 100 ml by addition of water and used the same day for creatine kinase assays. All reagents were from Boehringer Mannheim GmbH. The samples were stored at + 4 °C and assayed on the day of collection.

Results

65 samples with creatine kinase activity above 40 U/l were assayed with all 3 methods.

Figure 1 shows the excellent correlation of yeast glucose-6-phosphate dehydrogenase/$NADP^+$ versus *leuconostoc* glucose-6-phosphate dehydrogenase/$NADP^+$.

Figure 2 shows the relative differences of the methods as a function of creatine kinase activity obtained with method 1 (yeast glucose-6-phosphate dehydrogenase/$NADP^+$).

In table 1 the differences found are tabulated.

Figure 3 gives the correlation between yeast glucose-6-phosphate dehydrogenase/$NADP^+$ versus *leuconostoc*

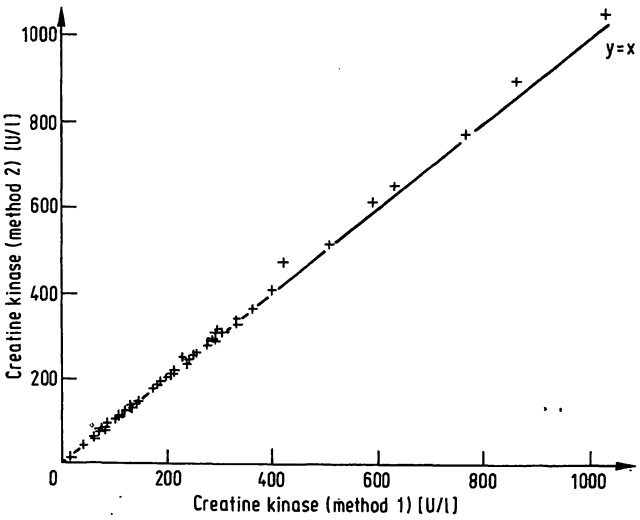

Fig. 1. Diagram of pairs of measured values.
Method 1: yeast glucose-6-phosphate dehydrogenase 1.73 kU/l assay volume. $NADP^+$: 2 mmol/l.
Method 2: *leuconostoc* glucose-6-phosphate dehydrogenase: 1.73 kU/l assay vomume. $NADP^+$: 2 mmol/l.

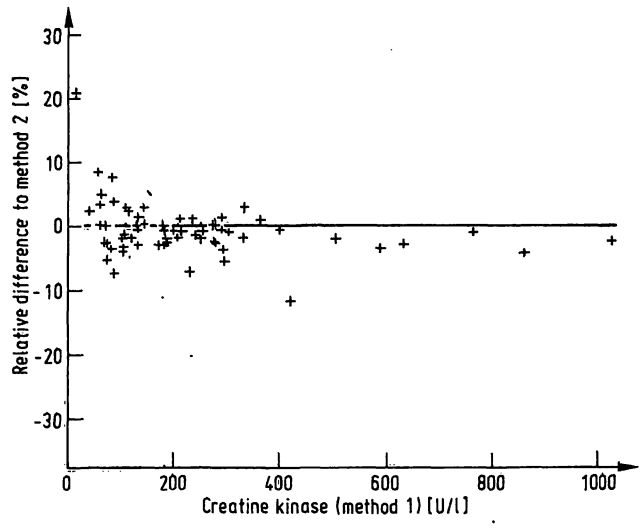

Fig. 2. Relative differences of methods.
(method 1 – method 2) · 100/method 1 as related to shown glucose-6-phosphate dehydrogenase activities found with method 1.
Method 1: yeast glucose-6-phosphate dehydrogenase 1.73 kU/l assay volume. $NADP^+$: 2 mmol/l.
Method 2: *leuconostoc* glucose-6-phosphate dehydrogenase 1.73 kU/l assay volume. $NADP^+$: 2 mmol/l.

Tab. 1. Differences found between method 1 and method 2.

	Relative Differences of methods $\frac{(\text{method 1} - \text{method 2}) \cdot 100}{\text{method 1}}$	Absolute differences of methods (method 1 – method 2)
Mean value	– 0,8 %	– 4,3 U/l
$\text{Difference}_{min.}$	–12,0 %	–51,0 U/l
$\text{Difference}_{max.}$	20,7 %	9,5 U/l

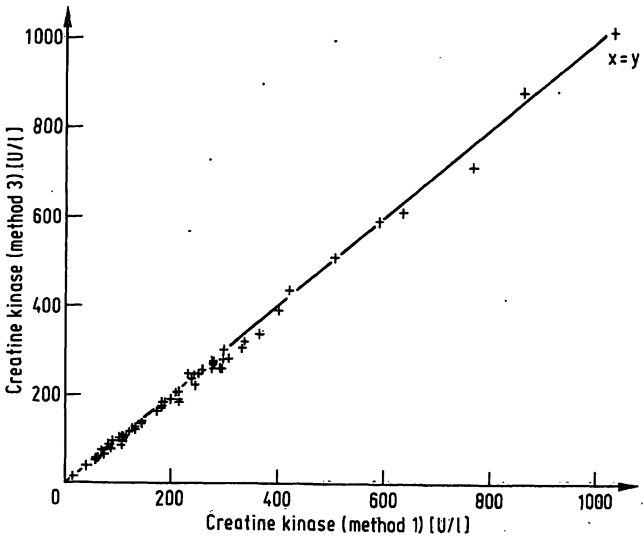

Fig. 3. Diagram of pairs of measured values.
Method 1: yeast glucose-6-phosphate dehydrogenase 1.73 kU/l assay volume. $NADP^+$: 2 mmol/l.
Method 3: *leuconostoc* glucose-6-phosphate dehydrogenase: 1.73 kU/l assay volume. NAD^+: 2 mmol/l.

Tab. 2. Differences found between method 1 and method 3.

	Relative Differences of methods $\frac{(\text{method 1} - \text{method 3}) \cdot 100}{\text{method 1}}$	Absolute differences of methods (method 1 – method 3)
Mean value	5,8 %	10,0 U/l
$\text{Difference}_{min.}$	– 5,6 %	–19,0 U/l
$\text{Difference}_{max.}$	23,1 %	53,0 U/l

glucose-6-phosphate dehydrogenase/NAD^+. It is seen that with the NAD^+-system lower values result as compared to $NADP^+$. These relative differences of methods are shown in figure 4 as a function of method 1 (yeast glucose-6-phosphate dehydrogenase/$NADP^+$). They are tabulated in table 2.

Relative differences between method 1 and 2 for all – except two values – are < 10% with a mean value of –0.8% only, whereas relative differences between method 1 and 3 scatter widely from – 5 to + 20% with a mean value of 5.8% higher activity of the yeast glucose-6-phosphate dehydrogenase/$NADP^+$-system. This magnitude of difference in methods using NAD^+ compared to methods with $NADP^+$ was expected and is attributable to interfering enzymes in serum, e.g. lactate dehydrogenase. We therefore recommend the use of $NADP^+$ with either yeast or *leuconostoc* glucose-6-phosphate dehydrogenase for assays of creatine kinase in serum.

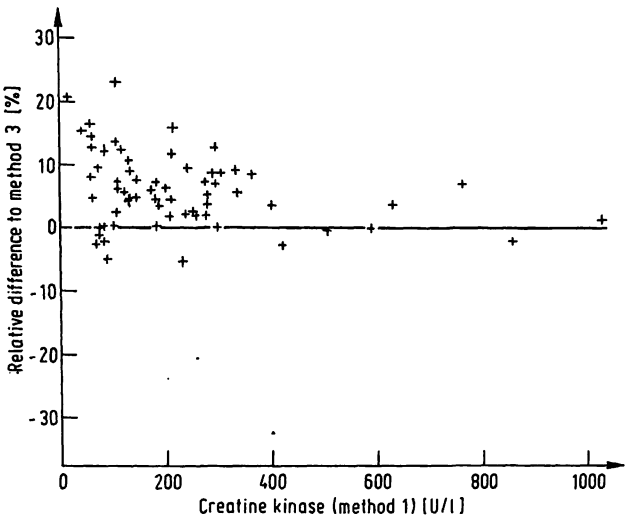

Fig. 4. Relative differences of methods (method 1–method 3) · 100/method 1 shown as related to activities found with method 1.

Method 1: yeast glucose-6-phosphate dehydrogenase 1.73 kU/l assay volume $NADP^+$: 2 mmol/l.
Method 3: *leuconostoc* glucose-6-phosphate dehydrogenase: 1.73 kU/l assay volume NAD^+: 2 mmol/l

Acknowledgement

The assistance of *S. Looser* in preparing the experiments and of *D. Markowetz* for the summary of results is gratefully acknowledged.

References

1. Szász, G., Gruber, W. & Bernt, E. (1976). Clin. Chem. *22*, 650–656.
2. Szász, G., Gerhardt, W., Gruber, W. & Bernt, E. (1976). Clin. Chem. *22*, 1806–1811.
3. Szász, G., Gerhardt, W. & Gruber, W. (1977). Clin. Chem. *23*, 1888–1892.
4. Szász, G. & Gruber, W. (1978). Clin. Chem. *24*, 245–249.
5. Szász, G., Gerhardt, W. & Gruber, W. (1978). Clin. Chem. Clin. Chem. *24*, 1557–1563.
6. Szász, G., Waldenström, J. & Gruber, W. (1979). Clin. Chem. *25*, 446–452.
7. Recommended method for the determination of creatine kinase in blood. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Scand. J. Clin. Lab. Invest. *36*, 711 (1976).
8. Recommendations pour la mesure de l'activite catalytique de la creatine kinase dans le serum humain a 30 °C. Commission "Enzymologie" of the Comite de Standardisation of the Societe Française de Biologie Clinique. Ann. Biol. Clin. *34*, 297 (1976).
9. Recommendations of the German Society for Clinical Chemistry. Standardization methods for the estimation of enzyme activities in biological fluids. Standard method for the determination of creatine kinase activity. Revised draft of 1976. J. Clin. Chem. Clin. Biochem. *15*, 255–260 (1977).
10. Aanbevolen methode voor het meten van de activiteit van kreatine kinase (CK) in serum. Enzymcomissie of the Nederlandse Vereniging voor Klinische Chemie. Mededelingen *3*, 220 (1978).
11. Empfohlene Methoden zur Bestimmung von 6 Enzymen im Blutplasma: GOT, GPT, LDH, ALP, CPK und GGT. Fachkommission der Schweizerischen Gesellschaft für Klinische Chemie. Bull. Schweiz. Ges. Klin. Chem. *1978*, p. 15.
12. Noldmann, E. A. & Kuby, S. A. (1963) Enzymes *7*, 223–242.
13. De Moss, R. D. (1955) Enzymol. *1*, 328–334.
14. Da Fonseca-Wollheim, F. (1973) Z. Klin. Chem. Klin. Biochem. *11*, 426–431
15. Szász, G., Busch, E. W., Koller, P. U. & Vollmar, J. (1976). Clin. Chem. *22*, 1213.
16. Gruber, W. (1978) Clin. Chem. *24*, 177–178.

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